Tropical Medicine and International Health doi:10.1111/tmi.13071

VOLUME 23 NO 7 PP 765–773 JULY 2018

Haematological and immunological reference intervals for adult population in the state of Amhara, Ethiopia

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Abstract

OBJECTIVE Reference intervals (RIs) currently being used in Ethiopia are derived from western populations. Thus, this study aimed to establish locally derived haematological and immunological RIs.

METHOD The study was conducted in Amhara State, Ethiopia with a total of 967 (55.2% males) participants. 56.9% of males and 43.1% of females were eligible for haematological and immunological RI determination. A non-parametric test was used for the determination of upper (97.5th percentile) and lower (2.5th percentile) reference interval limits with 95% CI. The Harris and Boyd Rule was used to determine the need of partitioning of reference intervals based on gender.

RESULT The established 95% reference intervals (2.5th–97.5th percentile) were: for WBC: 3–11.2 $\times$ 10^9/l; for platelet: 90–399 $\times$ 10^9/l; for RBC: 4–6 $\times$ 10^{12}/l for males and 3.5–5.6 $\times$ 10^{12}/l for females; for haemoglobin: (Hgb) 12–18.9 g/dl for males and 10.7–17.5 g/dl for females; for PCV: 35.7–55.3% for males and 32.2–50.1% for females; for CD4: 400–1430 $\times$ 10^9/l for males and 466–1523 $\times$ 10^9/l for females; for CD4 percentage: 18–49.1% for males and 21.3–52.9% for females; for MCV: 81–100 fl; for MCH: 25.3–34.6 pg; MCHC: 28.8–36.9%; for RDW: 11.6–15.4% and for MPV: 8–12.3 fl. Males had significantly higher RBC, Hgb and PCV than females. CD4 counts and CD4 percentage were significantly higher in females.

CONCLUSION The reference intervals established in this study differ from others and thus should be used for the interpretation of laboratory results in diagnosis and safety monitoring in clinical trials in Amhara.

keywords reference interval, haematological parameter, immunological parameter, Amhara

Introduction

Reference intervals (RIs) are also popularly known as reference ranges, normal values, normal ranges, biological reference intervals and expected values [1]. In clinical practice, before physiological assessments, medical diagnosis and management decisions are made, patient's laboratory results are compared with the corresponding reference intervals (RIs), which are bounded by a pair of reference limits [1, 2]. RIs aid physicians in decision-making in clinical medicine to interpret results, in evaluating the state of health of individuals and populations, in identifying people at risk for disease, in assessing immune status, disease progression and treatment responses [3]. They may also be used in clinical trials as a guide to setting inclusion or exclusion criteria and form the basis for safety monitoring of trial participants [4].

Laboratory parameters vary considerably between healthy people from different geographical locations, mostly driven by ethnic, genetic, demographic, nutritional, economic and environmental differences [5–10]. Moreover, they vary from laboratory to laboratory due to differences among laboratories in clinical service needs, analytic platforms, set criteria to define populations of healthy individuals, analytic imprecisions made when RIs were determined, differences in methods used to establish RIs, techniques and timing of blood collection, subject's posture when the sample is taken, and physical activity of individuals [11–13].

Despite these differences, most African countries including Ethiopia use western population-derived laboratory RIs for clinical diagnosis and research activities. Studies conducted to evaluate and establish locally derived RIs in several African countries [6, 7, 14–22] revealed significant differences of locally derived RIs from those of currently used western population-derived RIs. This may lead to misclassification of patients that may result in misdiagnoses, improper treatments or both. Unless RIs are representative of the patient's demographics and determined using similar pre-analytical procedures and comparable analytical methods, they are inappropriate as a diagnostic reference for clinical decision-making.

Several factors affect haematological and immunological parameters. Therefore, the Clinical Laboratory Standards Institute/International Federation for Clinical Chemistry (CLSI/IFCC) recommends that each laboratory establish RIs from the local population [5, 10, 12]. Although some attempts have been made to determine RIs in Ethiopia, they are fragmented and specific to small groups [14, 15], not comprehensive enough to be used in clinical practice [16, 22–24] and may lead to wrong interpretation [25]. The RIs currently being used in Ethiopia are derived from Caucasian populations.

Therefore, we aimed to establish locally derived RIs of haematological and immunological parameters for adults in the state of Amhara, Ethiopia. This the first study in the country covering a large population.

Methods

Study setting and design

This was a cross-sectional study of 1040 blood donors in the Amhara blood bank districts of Gondar, Bahirdar, Debre Markos and Dessie to determine haematological and immunological RIs. Amhara (9° to 13° 45′ N and 36° to 40° 30′E; 500–4620 m asl, 159 173.66 km^2) comprises hot dry tropical, sub-tropical, temperate and alpine climatic zones. In 2007, Amhara had a population of 17.2 million; 51.9% of the Ethiopian population were aged 15–64 years and 45% were children <15 years [26].

Selection of reference population

We used a convenience sampling technique to select the reference population during blood donation. Each blood bank has a fixed blood collection centre and mobile blood collection sites. The mobile blood collection teams campaign in the community and collect blood from volunteers. The objective of the study was explained to the donors and informed consent taken. Volunteers' general good health was assessed through a medical history and physical examination. Donors were screened for infectious diseases by the Ethiopian blood bank service after blood donation. We excluded individuals known to have diabetes mellitus, chronic renal insufficiency, hypertension, ischemic heart disease, anaemia, thyroid or liver disease; those taking pharmacologically active substances or any prescription drugs; smokers; individuals who had malaria in the previous 3 months, individuals who had jaundice or major surgery in the past year; pregnant (determined clinically or by urine HCG test) and lactating women; individuals who had donated blood in the previous 4 months and those who had received a blood transfusion in the previous year. Serum blood samples were screened for HIV, Hepatitis B virus, Hepatitis C virus and syphilis using ELISA techniques at each blood bank centre.

Laboratory analysis

Four ml of blood was collected from the antecubital vein into a K3EDTA vacutainer tube for CD4 and complete blood count. A three-part differential complete blood count (CBC) was analysed using CELL-DYN® 1800 (Abbott Laboratories Diagnostic Division, USA) at each blood bank centre. We used diluent, detergent and CN-free diff lyse CELL-DYN reagents supplied by Abbott Laboratories Diagnostic Division. The analyser used electrical impedance to count and size blood cells and the methaemoglobin method for determination of Hgb, based on the measurement of changes in electrical resistance produced by cells suspended in conductive diluents as they pass through an aperture of known dimensions. As each cell passes through the aperture, a transitory change in the resistance between the electrodes occurs, producing a measurable electrical pulse. The number of pulses generated indicates the number of cells and the amplitude of each pulse is proportional to cell volume. In this manner cells are counted and classified into white blood cells (WBC), red blood cells (RBC) and platelets. Haemoglobin (Hgb), packed cell volume (PCV), absolute and relative number of lymphocytes, neutrophils and mixed cells (basophils, monocytes, eosinophils), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were analysed.

CD4 cell counts and CD4 percentages were determined using a Becton Dickinson Fluorescence Activated Cell Sorting (BD FACS) Count™ system (Becton Dickinson, San Jose, CA, USA), which provides reproducible and accurate results even with low CD4 counts and is widely used for CD4 monitoring in HIV/AIDS treatment programs. BD FACS Count reagents are provided as

complete kits that streamline CD4 counting. These kits contain ready-to-use tubes with pre-measured antibodies and beads for absolute counting, fixative solution, and software that enables automated analysis without operator intervention. The beads and lymphocytes of interest are automatically identified and absolute counts (cells/μL) and percentages calculated. Quality controls in the software detect and flag error conditions and suppress results when control limits are exceeded.

Quality control

Protocols for sample collection, processing and transportation were strictly followed to have safe procedures and reliable specimens. Samples were analysed in batches within 2 h of collection and delayed samples were kept at 2–8 °C for a maximum of 12 h. During analysis, both internal and external quality assurance (QA) protocols were followed. As an internal QA, commercial quality control samples were included in every batch. Test samples were run if and only if quality control samples were within range. For haematology tests three levels of whole blood controls (High, Medium, and Low) and for CD4 cell count, two levels of whole blood controls (Low, Normal) were used. All analytes were analysed by following standard operational procedures. As an external QA, the laboratory received quality control samples from the central laboratory, analysed them as a patient sample and returned them to the centre. The central laboratory then analysed the data and gave performance evaluation feedback to each laboratory. The central laboratory received QA materials from UK and Canada.

Statistical analysis

Data were checked for completeness, cleaned, edited and entered into Epi-Info version 3.5.1 and then transported to SPSS v. 20 for statistical analysis. Outliers within each subgroup were identified using the Dixon and Reed method, namely the D/R ratio, where D is the absolute difference between an extreme observation (large or small) and the next largest (or smallest) observation, and R is the range (maximum–minimum). Extreme values were deleted from the distribution if $D/R \geq 1/3$ [5]. RIs were calculated according to the guideline of the Clinical Laboratory Standards Institute/International Federation for Clinical Chemistry (CLSI/IFCC) by employing a non-parametric method to determine the median interquartile ranges (IQR), combined or separate 95% RIs (2.5th and 97.5th percentiles). The 2.5th and 97.5th percentiles were considered as lower and upper reference limits, respectively, covering 95% of the reference interval of each parameter [5]. RIs were determined separately for males, females and combined genders. To determine combined or separate gender RIs, the Harris and Boyd test was used as described below [27, 28]:

$$Z = \frac{\text{Mean 1} - \text{Mean 2}}{(\text{SD1}/N1 + \text{SD2}/N2)^{1/2}}$$

where SD is standard deviation and N is the number of samples of each gender. This statistical Z result was compared with a critical Z^* value:

$$Z^* = 3[(N1 + N2)/240]^{1/2}$$

Separate RI was determined when $Z > Z^*$. Based on this test, separate- gender RIs were determined for RBC, Hgb, PCV, CD4 and CD4 percentages (Table 1).

Ethics

This study was approved by the Research Review Board of University of Gondar and permission was obtained

Table 1 Result of Harris and Boyd test, which was performed to see the need for partitioning of reference interval based on gender

	Harris and Boyd		
Parameters	Z^*	Z	Decision
WBC ($\times 10^9$/L)	6.0	0.7	No separate RI
Neutrophil ($\times 10^9$/L)	6.0	1.1	No separate RI
Lymphocyte ($\times 10^9$/L)	6.0	0.1	No separate RI
Mixed cells ($\times 10^9$/L)	6.0	0.6	No separate RI
Neutrophil (%)	6.0	1.2	No separate RI
Lymphocyte (%)	6.0	0.3	No separate RI
Mixed cells %	6.0	2.0	No separate RI
Platelet ($\times 10^9$/l)	6.0	3.3	No separate RI
RBC ($\times 10^{12}$/l)**	6.0	18.6	Separate RI
Hgb (g/dl)**	6.0	16.7	Separate RI
PCV (%)**	6.0	19	Separate RI
MCH (pg)	6.0	1.3	No separate RI
MCHC (g/dl)	6.0	0.6	No separate RI
MCV (fl)	6.0	1.7	No separate RI
RDWCV	6.0	3.4	No separate RI
MPV	6.0	1	No separate RI
CD4 ($\times 10^9$/l)**	5.4	6.2	Separate RI
CD4 percentage**	5.4	6.7	Separate RI

Z^*, critical value, Z, calculated value, Separate RI are needed only when Z is greater than Z^*; **Indicate haematological and CD4 parameters which need partition between male and female ($Z > Z^*$).

from the blood banks. Written informed consent was obtained from each participant. The study participants were informed about the aim of the study and its procedure, and inclusion was voluntary. Confidentiality was kept and withdrawal was possible at any stage.

Results

We enrolled 1040 volunteer blood donors aged 18–61 years. 55.4% were male and 44.6% female. Of the total, 967 (55.2% males and 44.8% females) were eligible for haematological RI determination and 784 (56.9% males and 43.1% females) were eligible for immunological RI determination. The median age of study participants was 20 years with an IQR of 5 years. 80% were students aged 18–25 years.

The median and 95% reference intervals (2.5–97.5th percentiles) for haematological and immunological parameters are shown in Table 2. The combined median and 95th percentile for both gender s were as follows: 6.1 (3–11.2 $\times$ 10^9/l) for WBC, 3.2 (1.1–6.7 $\times$ 10^9/l) for absolute neutrophils, 2.1 (1.1–4.5 $\times$ 10^9/l) for absolute lymphocytes, 0.7 (0.2–2.1 $\times$ 10^9/l) for absolute mixed cells, 239 (90–399 $\times$ 10^9/L) for platelet, 90.1 (81–100 fl) for MCV, 30 (25.3–34.6 pg) for MCH, 33.9 (28.8–36.9%) for MCHC, 13.2 (11.6–15.4%) for RDW and 10 (8–12.3 fl) for MPV for both genders.

The separate RIs for males were 5.1 (4–6 $\times$ 10^{12}/l) for RBC, 15.3 (12–18.9 g/dl) for Hgb, 46.2 (35.7–55.3%) for PCV, 721 (400–1430 $\times$ 10^9/l) for CD4 and 36.3 (18–49.1%) for CD4 percentage. RIs for females were 4.5 (3.5–5.6 $\times$ 10^{12}/l) for RBC, 13.1 (10.7–17.5 g/dl) for Hgb, 39.9 (32.2–50.1%) for PCV, 830 (466–1523 $\times$ 10^9/L) for CD4 and 40.3 (21.3–52.9%) for CD4 percentage. Males had significantly higher RBC, Hgb and PCV than females, whereas CD4 counts and CD4 percentages were significantly higher in females. The distribution of these variations with gender are expressed in Box and Whisker plots (Figure 1).

Discussion

Local RIs are recommended to be established and used for interpretation of laboratory results during screening,

Table 2 Median, IQR, and 95th percentile of reference interval with lower and upper limit 95% CI of haematological and immunological parameters

Parameter	*N*	Median (IQR)	RI (95th percentile)	Lower limit 95% CI	Upper limit 95% CI
WBC ($\times 10^9$/l)	967	6.1 (4.8–7.7)	3–11.2	3 (2.7–3.1)	11.2 (10.8–11.9)
Neutrophil ($\times 10^9$/l)	967	3.2 (2.2–4.2)	1.1–6.7	1.1 (0.9–1.2)	6.7 (6.5–6.9
Lymphocyte ($\times 10^9$/l)	967	2.1 (1.7–2.6)	1.1–4.5	1.1 (1–1.2)	4.5 (4.2–4.7)
Mixed cells ($\times 10^9$/l)	967	0.7 (0.5–1)	0.2–2.1	0.2 (0.2–0.3)	2.1 (2–2.1)
Neutrophil %	967	52 (43.5–59.4)	27.2–71.9	27.2 (25.4–29.9)	71.9 (69.6–73.1)
Lymphocyte %	967	35.4 (29.4–42.1)	19.8–57.4	19.8 (18.8–20.9)	57.4 (56–58.1)
Mixed cells %	967	11.3 (8.9–15.2)	5.2–26.4	5.2 (4.5–5.6)	26.4 (25.2–28
PLT ($\times 10^9$/l)	967	239 (186–293)	90–399	90 (84–94)	399 (381–417)
RBC ($\times 10^{12}$/l)					
Male	534	5.1 (4.8–5.5)	4.0–6.0	4 (3.9–4.2)	6 (6–6.2)
Female	433	4.5 (4.2–4.8)	3.5–5.6	3.5 (3.4–3.6)	5.6 (5.4–5.9)
Hgb (g/dl)					
Male	534	15.3 (14.2–16.5)	12–18.9	12 (11.6–12.2)	18.9 (18.5–19.3)
Female	433	13.1 (12.1–14.3)	10.7–17.5	10.7 (10.5–11)	17.5 (17–18.3)
PCV (%)					
Male	534	46.2 (43.6–49.2)	35.7–55.3	35.7 (34.8–36.9)	55.3 (54.2–56)
Female	433	39.9 (37.6–43.5)	32.2–50.1	32.2 (31–33)	50.1 (48.5–52.4)
MCV (fl)	967	90.1 (87–93)	81–100	81 (80.4–82)	99.7 (98.7–100.7)
MCH (pg)	967	30 (28.1–31.7)	25.3–34.6	25.3 (25.1–25.7)	34.5 (34.3–35.2)
MCHC (%)	967	33.9 (30.5–35.1)	28.8–36.9	28.8 (28.5–29)	36.9 (36.6–37.1)
RDW (%)	967	13.2 (12.7–13.8)	11.6–15.4	11.6 (11.4–11.8)	15.4 (15.2–15.7)
MPV (fl)	967	10 (9.3–10.6)	8–12.3	8 (7.9–8.2)	12.3 (12–12.4)
CD4 ($\times 10^9$/l)					
Male	446	715 (592–874)	400–1406	400 (370–431)	1406 (1250–1477)
Female	338	830 (677–1017)	466–1523	465 (388–494)	1523 (1412–1621)
CD4%					
Male	446	36.3 (31.8–40.7)	18–49.1	18 (15.7–20.6)	49.1 (47.9–49.9)
Female	338	40.3 (34.6–44.7)	21.3–52.9	21.3 (15.9–25.6)	52.9 (51.1–55.4)

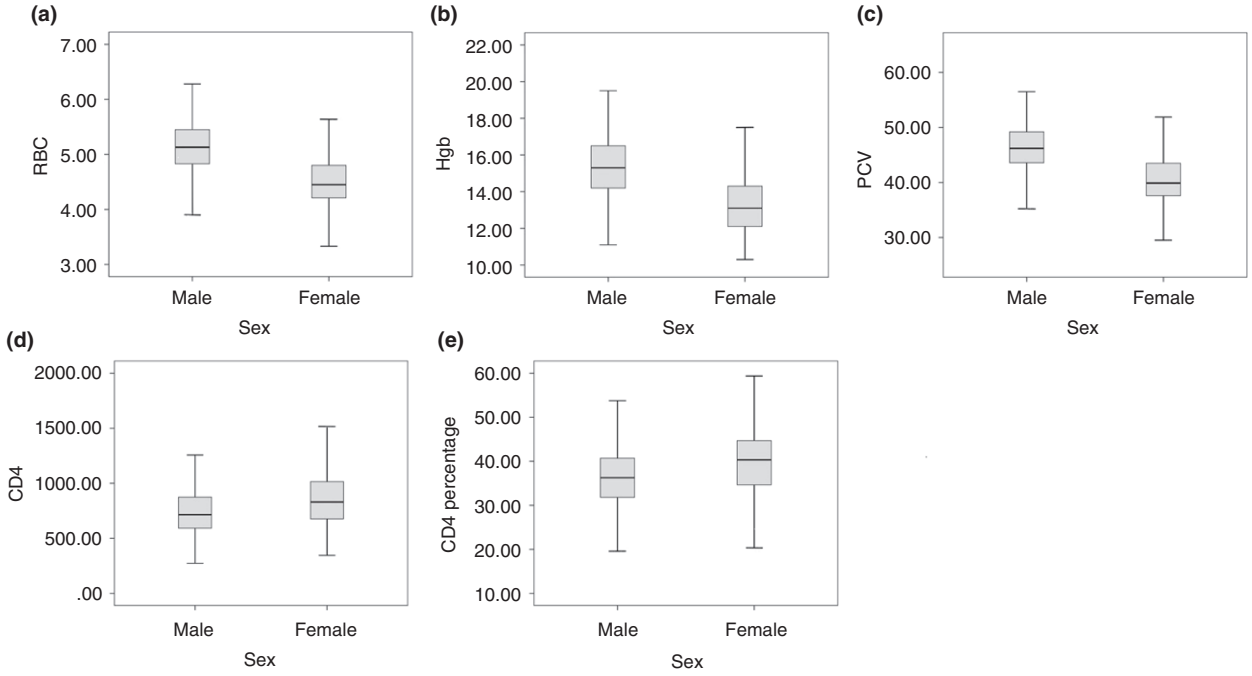

Figure 1 Box and whisker plots indicating the effect of gender on haematological and immunological parameters of the study participants. (a) Red blood cells, (b) Haemoglobin, (c) Packed cell volume, (d) CD4 counts and (e) CD4%.

diagnosis and monitoring of patients and to assure the health status of individuals involved in clinical trials [4–6, 12]. The RIs derived in this study varied from those reported in other areas [6, 14, 15, 29–31] and in text books [12, 32–34], as would be expected for populations in other geographical locations, with genetic and dietary diversities [5]. As indicated in Table 3, the lower RI limit of total WBC count in this study was lower than previously reported from different parts of Ethiopia [14, 15], Eastern and Southern Africa [6], Western Kenya [7], Ghana [29], USA [30] and Caucasian populations [12, 32, 33]; but it was higher than in Kericho district of Kenya [31], Togo [35] and Uganda [4]. The WBC RI for this study is in agreement with southern Tanzania's [36] RI lower limit, whereas our upper RI limit was lower than in Gojjam district of Ethiopia [15], in books [32, 33] but higher than reported from Africa [4, 6, 7, 14, 31, 35], USA [30] and in text books [12, 34]. Similarly the absolute neutrophil and lymphocyte count RI upper limits are higher than in most African countries RIs [4, 6, 7, 14, 31, 35] but lower than in Caucasian populations [12, 30, 34]. These variations are usually associated with well-known differences in altitude, environment, diet and ethnic background [5]. There was no significant difference in WBC count between males and females, confirming results of other studies [6, 14, 15, 29–31].

The 95th percentile RIs of our lower limit RI of RBC in both genders were lower than those in Kenya [7, 31], Tanzania [36], USA [30] and western text books [32, 34] and higher than in previous studies in Gondar, Ethiopia [14], Ghana [29], Togo [35] and Uganda [4]. The upper limit RI was lower than previous studies in Ethiopia [14, 15], Togo [35] but higher than in Ghana [29], USA [30] and in western books [32, 34]. The differences might be due to geographical variation (altitude), environment, diet, ethnic background, method and instrument used for analysis (CLSI) [5, 17].

The lower limit of Hgb and PCV RIs in this study was lower than in Gojjam [15], Tanzania [36] and the USA [30], but higher than in Eastern and Western Africa [6], Ghana [29], Western Kenya [7], Togo [35] and Uganda [4] for both genders. The upper limit of Hgb was higher than in Eastern and Western Africa [6], Ghana [29], Kenya [7, 31], Togo [35], Uganda [4], USA [30] but lower than in Gojjam [15], probably due to altitude and ethnic variations [5, 37, 38]. Thus, it may not be possible to even have one standard reference between two localities in the same county, as seen in Ethiopia and Kenya [7, 14, 15, 31].

The reference intervals of MCV, MCH and MCHC in this study varied from those of other studies (Table 3). Generally, the RI of MCV is higher than in other African

Table 3 Comparison of haematological RIs of the current study with previous studies in Africa, USA and test books

Parameters	Current Study	Gondar, Ethiopia [14]	Gojjam, Ethiopia [15]	Kericho, Kenya [31]	Eastern & Southern Africa [6]	Ghana [29]	Southern Tanzania [36]	Uganda [4]	Togo [35]	USA [30]	Rodak's Haematology [34]
WBC ($\times 10^9$/l)	3–11.2	3.2–8.8	3.5–11.5	2.8–8.2	3.1–9.1	3.4–9.2	3–7.9	2.8–8.2	1.9–10.1	4.5–11	3.6–10.6
Neutrophil ($\times 10^9$/l)	1.1–6.7	1.6–5.1	NA	0.9–4.7	1.0–5.3	1.5–5.6	1.1–4.7	0.9–3.9	0.5–5.4	NA	1.7–7.5
Lymphocyte ($\times 10^9$/l)	1.1–4.5	1–3.5	NA	1.1–3.5	1.2–3.5	1.2–4.4	1.1–3	1.2–3.7	1.1–4.3	NA	1–3.2
Mixed cells ($\times 10^9$/l)	0.2–2.1	0.2–1	NA	NA	NA	NA	0.3–1.1	NA	NA	NA	NA
Neutrophil %	27.2–71.9	36–69	34.3–72.1	20–60	25–66	32–68.1	32–69.1	22.2–59.3	NA	40–70	50–70
Lymphocyte %	19.8–57.4	22–55	17.9–53.4	20–60	23–59	25.2–57.7	20.8–56.7	26.7–61.2	NA	22–44	18–42
Mixed cells %	5.2–26.4	6–13	6–23	NA	NA	NA	5.6–19.8	NA	NA		NA
PLT ($\times 10^9$/l)	90–399	128–432	113.9–372	120–411	126–438	89–380	150–395	109–384	120–443	150–350	150–450
RBC ($\times 10^{12}$/l)											
M	4.0–6.0	3.53–6.93	3.9–6.2	4–6.4	4.4–6.3	3.79–5.96	4.41–6.27	3.8–6.1	3.3–6.4	4.5–5.9	4.2–6
F	3.5–5.6	3.45–6.25	3.9–5.8	3.8–5.6	3.7–5.6	3.09–5.30	3.84–5.59	3.3–5.3	3.1–6	4–5.2	3.8-5.2
Hgb (g/dl)											
M	12–18.9	11.5–18	13.6–19.8	12.2–17.7	8.3–11.3	11.3–16.4	13.7–17.7	11.6–17.1	10–18.4	13.5–17.5	13.5–18
F	10.7–17.5	10.9–16.9	12–18	9.5–15.8	5.9–10	88–144	11.1–15.7	9.8–16.2	10.3–17.1	12–16	12–15
PCV (%)											
M	35.7–55.3	36.2–58.6	45–59	35–50.8	40–50	33.2–50.5	40.2–53.7	33.8–49.5	28–54	41–53	40–54
F	32.2–50.1	32.1–56.6	39–55	9.4–45.4	30–50	26.4–45.0	36.2–46.8	28.3–46.8	28–47	36–46	35–49
MCV (fl)	81–100	85–100	89.5–107.5	68.8–97.2	68–98	72–97	77.6–98.1	71–97	80–99	80–100	80–100
MCH (pg)	25.3–34.6	NA	28–34	22.4–33.5	NA	22.6–33.5	23.6–33.1	23.5–33.7	25–37	26–34	26–34
MCHC (%)	28.8–36.9	NA	30–33.2	32.2–35.3	NA	30.5–36.2	30.6–34.9	32.5–35.3	29–41	31–37	32–36
RDW (%)	11.6–15.4	12–18	NA	NA	NA	12.6–23	NA	11–16.8	NA	11.5–14.5	11.5–14.5
MPV (fl)	8–12.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	7–12

N.B: NA; not applicable (there is no value).

countries [4, 6, 7, 29, 31, 36] and comparable with Togo [35], USA [30] and values in text books [34]. MCH and MCHC values showed variable results and RDW RIs are comparable with other studies [30, 34].

We observed a significant gender difference on red cell parameters (RBC, Hgb and PCV). Higher values were seen in males than females, confirming previous findings [12, 32–34] in Ethiopia [14, 15], Eastern and South Africa [6], Ghana [29], Kenya [7, 31], South Tanzania [36], Togo [35], Uganda [4] and USA [30]. The significant differences between men and women in RBC, Hgb and PCV may be due to biological and physiological factors, such as the influence of androgen hormone on erythropoiesis [39] and menstrual blood loss in females [40].

The RIs of platelets in this study were lower than in Gondar [14], Eastern and Southern Africa [6], Kenya [7, 31], Togo [35] and Caucasian populations [32, 34]. However, the RI values are higher than Ghana [29]. This is may be associated with high altitude in Ethiopia, as platelet count decreases and mean platelet volume (MPV) increases with altitude [41–43] (Table 4).

CD4 RI determination is also important because CD4 lymphocyte cells are used for assessment of immune status, disease progression and treatment responses of patients, especially individuals living with HIV [3, 44]. CD4 cell counts were higher than in Addis Ababa [16], Tanzania [21] and Central African Republic [20]; but lower than in Gojjam, Ethiopia [15], Gauteng, South Africa [45] and Turkey [46]. CD4 percentages RI in this study were generally lower than in other countries [7, 16, 45, 46], possibly due to ethnic and environmental factors [47, 48]. There was a significant gender difference in absolute CD4 cells and CD4 percentages. The higher values seen in women in our study are consistent with other studies [7, 15, 16, 19, 20, 45] and may be due to the effect of sex hormones [49].

One limitation of our study is that the majority of the reference population included were young adults. Secondly, we did not screen the reference population for infectious diseases such as intestinal parasites.

Table 4 Comparison of CD4 and CD4% RIs of the current study with previous studies in Ethiopia, other African countries and Turkey

Parameters	Current Study	Gojjam, Ethiopia [15]	Addis Ababa, Ethiopia [16]	Western Kenya [7]	Central African Republic [20]	Northern Tanzania [21]	Uganda [19]	Gauteng, South Africa [45]	Turkey [46]
CD4 ($\times 10^9$/L)									
M	400–1430	414–1474	306–1249	462–1306	391–1145	362–1376	503–1807	291.2–1278.9	438–1890
F	466–1523	436–1695	456 –1368	440–1602	386–1355	454–1485	561–2051	288.5–1406.8	394–2234
CD4%									
M	18–49.1	NA	NA	32–55	23–54	16.5–45.3	27.7–56.7	NA	29.91–60.12
F	21.3–52.9	NA	NA	29–54	29–55	22.5–48.3	31.6–58.0	NA	29.74–61.41

Conclusion

The reference values we established for the state of Amhara, Ethiopia will be helpful for the screening, diagnosis, monitoring and follow-up of patients. This study provides the first haematological and immunological RIs for a larger population in the state of Amhara, Ethiopia, and is the largest in the country. The RIs generated in Amhara differ from others in Africa and western countries. The WBC RI showed a wider range, with lower

and upper RI limits showing variations from previous studies. Similarly, RBC parameters (RBC, Hgb and PCV), showed a slightly higher upper RI limit, whereas platelet counts, absolute CD4 counts and CD4 percentages gave lower RIs in this study. Thus the use of western RIs could lead to wrong diagnosis and interpretation of laboratory results. It is also advisable to establish RIs for the younger and older populations, giving due consideration to the effect of infectious diseases on haematological and immunological parameters.

Acknowledgements

The authors thank the study participants for volunteering to participate in this study; the staff of the Gondar, Bahirdar, Debre Markos and Dessie Blood Banks for their support during data collection and infectious disease screening; the University of Gondar hospital, Amhara Public Health Institute Laboratory, Debre Markos Hospital and Dessie Referral Hospital Laboratory staff who supported for haematological and CD4 laboratory analysis; and the University of Gondar for funding the project. The datasets analysed during the current study were available from the corresponding author upon request.

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